Art. XXXI. *Longevity of Seeds and Structure and Nature of Seed Coat.*

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(With Plates LXXIX.–LXXXI.).

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The subject for this paper was suggested to me by Professor Ewart some time after the publication of his work on Longevity of Seeds.[1] From his experiments he found that the majority of macrobiotic seeds belong to the order Leguminosae, and that the highest percentage of germination occurs among the so-called "hard" seeds, which require soaking in sulphuric acid or other treatment to make them swell up in water. In the appendix to the same paper Dr. White showed that the hardness is almost invariably due to the presence of a distinct cuticle of varying thickness. In the small seeds the cuticle alone seems to confer impermeability, but in the larger types the palisade cells are also responsible.

As a large number of the hard seeds are of economic value, it is advisable to devise some convenient method of softening them, and, further, the method must be such as will have no detrimental effect on the germination. This work includes also records of a number of tests made for the purpose of ascertaining the lengths of time during which various seeds retain their power of germinating. This part is merely a few supplementary records to those made and compiled by Prof. Ewart in 1908.

The work has been carried out in the Botanical Laboratory of the Melbourne University under the supervision of Professor Ewart, to whom I wish to express my thanks for his assistance and untiring interest. I take the opportunity of thanking Mr. H. Pye, of the Dookie Agricultural College, and the Agricultural Department, for supplies of seeds through the National Herbarium, Melbourne.

1 Proc. Roy. Soc. Victoria, vol. xxi., pt. i. (1908).

Longevity of Seeds.

After the publication of the above-mentioned paper on "Longevity of Seeds," an additional set of seeds, all over 16 years old, was received by Professor Ewart and passed on to me to be tested. I used in addition a number of seeds sent from time to time from the National Herbarium, Melbourne. These seeds varied in age from one to fifty-nine years.*

The above seeds had all been stored in packages, and were in a clean, dry condition. In one instance only, *Albizzia lophantha*, had the seeds remained in the soil. They were gathered from ground which had been cleared of the trees in 1887, so the seeds were at least 23 years old, and the majority were probably much older.

The method of testing the seeds was that found to be most satisfactory by Prof. Ewart in his work. The seeds were counted (one hundred being used for each test whenever material permitted), sown on moist blotting paper in small glass vessels, and kept in a germination chamber at 30 deg. C. The material was inspected constantly, growth of mould and attacks of bacteria being prevented by frequent washing and renewing of the blotting paper.

In the case of samples containing hard seeds, the following method was employed. Those seeds which swelled in water were removed, and their percentage of germination noted, the remaining hard seeds were treated with sulphuric acid to remove the cuticle; the duration of the treatment depending on the resistance of the coat. The seeds were placed in strong sulphuric acid for a certain time, then washed repeatedly in water and finally with dilute ammonia to remove all traces of acid. The number of seeds which swelled after each treatment, and their percentage of germination, were recorded in the list.

The following list is a record of all the seeds tested. The name appears in the first column, the age of the seed in the second, the number of seeds used for the test in the third, the percentage of germination in the fourth, and in the fifth is noted the method of treatment. Where the name is marked with an asterisk all the observations are from the same sample of seed.

* Through the kindness of Prof. Vines a sample of the seed of an unnamed *Acacia* sent to Sir John Herschel, in 1843, was received for testing. Possibly owing to the smallness of the sample none of the seeds proved to be germinable, for within the past three or four years, Prof. Vines informs me, Sir William Herschel was able to raise a few short lived seedlings from the same seed.

	Years old.	No. of Seeds.	Per cent. germ.	
Abies amabilis, Forb.	16	50	nil	Sw. water.
*Abrus precatorius, L.	over 14	11	nil	Sw. water.
ditto	over 14	4	nil	Sw. after 1 hr. in acid.
*Acacia acinacea, Lindl.	40	49	nil	Sw. water.
ditto	40	11	55	Filed to cause swelling in water.
*Acacia decurrens, Willd.	16	8	25	Sw. water.
ditto	16	48	85	Sw. after 2 hrs. in acid.
ditto	16	18	22	Sw. after $3\frac{1}{2}$ hrs. in acid.
ditto	16	19	95	Sw. after $5\frac{1}{2}$ hrs. in acid.
ditto	16	5	100	Sw. after $8\frac{1}{2}$ hrs. in acid.
ditto	16	1	100	Sw. after $8\frac{1}{2}$ hrs. in acid, and subsequent filing.
*Acacia melanoxylon, R. Br.	16	22	9	
ditto	16	26	8	Sw. water.
ditto	16	40	46	Sw. after 1 hr. in acid.
ditto	16	10	33	Sw. after 2 hrs. in acid.
ditto	16	2	50	Sw. after $3\frac{1}{2}$ hrs. in acid.
*Acacia Oswaldi, F.V.M.	16	12	nil	Sw. after $6\frac{1}{2}$ hrs. in acid.
ditto	16	2	50	Sw. water.
*Acacia pycnantha, Benth.	16	7	nil	Sw. after 2 hrs. in acid.
ditto	16	26	23	Sw. water.
ditto	16	51	80	Sw. after 2 hrs. in acid.
ditto	16	13	62	Sw. after $3\frac{1}{2}$ hrs. in acid.
ditto	16	3	100	Sw. after $5\frac{1}{2}$ hrs. in acid.
Negundo aceroides, Moench.	16	54	nil	Sw. after $8\frac{1}{2}$ hrs. in acid.
(Acer negundo, L.)	16	100	nil	Sw. water.
Acer rubrum, L.	23	2	100	Sw. water.

* All from the same sample.

	Years old.	No. of Seeds.	Per cent. Germ.	
*Albizzia (Acacia), lophantha, Benth.	23	3	100	Sw. water.
ditto	23	2	100	Sw. after 1 hr. in acid.†
ditto	23	1	100	Sw. after 5 hrs. in acid.
ditto	23	18	100	Sw. after 7 hrs. in acid.
ditto	23	22	100	Sw. after 12 hrs. in acid.
ditto	23	38	100	Sw. after 18 hrs. in acid.
ditto	23	2	100	Sw. after 40 hrs. in acid.
ditto	23	1	100	Sw. after 43 hrs. in acid.
ditto	23	2	100	Sw. after 45 hrs. in acid.
ditto	23	3	100	Sw. after 48 hrs. in acid.
ditto	23	5	100	Sw. after 70 hrs. in acid.
ditto	23	1	100	Sw. after 84 hrs. in acid.
ditto	23	3	100	Sw. after 93 hrs. in acid.
*Albizzia lophantha, Benth.	23	41	100	Sw. water. ‡
ditto	23	5	100	Sw. after $7\frac{1}{2}$ hrs. in acid.
ditto	23	1	100	Sw. after $8\frac{1}{2}$ hrs. in acid.
ditto	40	15	nil	Sw. after $10\frac{1}{2}$ hrs. in acid.
Althaea narbonnensis, Power	16	100	nil	Sw. water.
Angophora subvelutina, F.V.M.	16	11	nil	Sw. water.
Atriplex spongiosa, F.V.M.	4	100	38	Sw. water.
Avena pratensis, L.	4	100	25	Sw. water.
ditto	6	100	64	Sw. water.
ditto	6	100	37	Sw. water.
ditto	7	100	48	Sw. water.
ditto	5	100	46	Sw. water.

* All from the same sample.
† Temperature 11 deg. C.
‡ Temperature 30 deg. C.

	Years old.	No. of Seeds.	Per cent. Germ.	
ditto	5	100	33	Sw. water.
ditto	6	100	6	Sw. water.
ditto	15	20	nil	Sw. water.
Bauhinia acuminata, L.	16	200	nil	Sw. water.
Betula alba, L.	50	100	nil	Sw. water.
Brassica alba, Boiss.	21	5	nil	Sw. water.
Butea frondosa, Roxb.	14	25	nil	Sw. water.
Cajanus indicus, Spreng.	11	100	nil	Sw. water.
Callitris, rhomboidea, R. Br.	29	100	nil	Sw. water.
ditto	13	82	nil	Sw. water.
Callitris Muelleri, Bth. and Hook, f.	21	44	nil	Sw. water.
Callitris, robusta, R. Br.	30	100	nil	Sw. water.
ditto	12	70	nil	Sw. water.
ditto	59	100	nil	Sw. water.
Carex Pseudo-cyperus, L.	43	32	nil	Sw. water.
*Cassia glauca, Lam.	43	50	10	Sw. water.
ditto	43	10	10	Sw. after $\frac{1}{2}$ hr. in acid.
ditto	43	4	85	Sw. after 1 hr. in acid.
ditto	43	1	100	Sw. after 2 hrs. in acid.
ditto	16	34	nil	Sw. after 3 hrs. in acid.
Catalpa bignonioides, Walt.	16	50	nil	Sw. water.
Catalpa speciosa, Warder	16	95	nil	Sw. water.
Cedrela Toona, Roxb.	16	14	nil	Sw. water.
Celtis australis, L.	14	6	nil	Sw. water.
Ceratonia Siliqua, L.	14	19	nil	Sw. water.
ditto	43	100	nil	Filed.

* All from the same sample.

	Years old.	No. of Seeds.	Per cent. Germ.	
Chenopodium album, L.	33	100	nil	Sw. water.
Chenopodium chilense, Schrad.	59	50	nil	Sw. water.
Crepis foetida, L.	16	100	nil	Sw. water.
Cupressus sempervirens, L.	14	94	nil	Sw. water.
Cytisus canariensis, Steud.	14	6	50	Sw. water.
ditto	17	50	46	Filed.
Dodonaea viscosa, Jacq.	17	50	24	Sw. water.
Dolichos biflorus, L. (white var.)	15	50	nil	Sw. water.
Duranta plumieri, Jacq.	20	25	nil	Sw. water.
Erythrina indica, Lam.	15	7	43	Sw. water.
ditto	21	50	nil	Sw. water.
Erythrina speciosa, Andr.	16	50	8	Sw. water.
Eucalyptus Baileyana, F.v.M.	16	100	1	Sw. water.
Eucalyptus botryoides, Sm.	16	50	30	Sw. water.
Eucalyptus calophylla, R. Br.	16	100	14	Sw. water.
Eucalyptus corynocalyx, F.v.M.	16	100	68	Sw. water.
Eucalyptus diversicolor, F.v.M.	16	100	3	Sw. water.
Eucalyptus Maideni F.v.M.	3	50	82	Sw. water.
Eucalytpus miniata, A. Cunn.	16	100	1	Sw. water.
Euchlaena mexicana, Schrad.	15	50	nil	Absorbed water but remained hard for many weeks.
Euphorbia eremophila, A. Cunn.	10	100	nil	Sw. water.
Exocarpus	16	34	nil	Sw. water.
Fatsia japonica, Deene and Planch.	1	100	nil	Sw. water.
Foeniculum vulgare, Mill.	15	100	nil	Sw. water.
ditto	15	100	nil	Sw. water.
ditto	14	100	nil	Sw. water.

	Years old.	No. of Seeds.	Per cent. Germ.	
Fraxinus americana, L.	16	37	nil	Sw. water.
Fraxinus pubescens, Lam.	16	37	nil	Sw. water.
Fraxinus sambucifolia, Lam.	16	18	nil	Sw. water.
Fraxinus viridis, Bosc.	16	33	nil	Sw. water.
Gleditschia sinensis, Lam.	40	9	nil	Sw. water.
*Gleditschia triacanthos	40	2	nil	Sw. water.
	40	8	nil	Filed.
Gomortega nitida, Ruiz and Pav.	2	4	nil	Very hard outer covering, water took long time to penetrate.
Heterodendrum oleaefolium, Desf.	11	25	nil	Sw. water.
Hieracium stiriacum, Kern.	30	50	nil	Sw. water.
Hordeum (sativum), vulgare, L.	6	100	nil	Sw. water.
ditto	6	100	86	Sw. water.
ditto	38	25	nil	Sw. water.
ditto	6	100	3	Sw. water.
ditto	4	100	55	Sw. water.
Hypericum Androsaemum, L.	27	100	nil	Sw. water.
Juniperus bermudiana, L.	13	50	nil	Sw. water.
Juniperinus flaccida, Schlecht.	15	15	nil	Sw. water.
Kentrophyllum lanatum, L.	44	100	nil	Sw. water.
Ligustrum Stauntoni, D.C.	15	25	nil	Sw. water.
Linum (monadelphum), usitatissimum, L.	42	100	nil	Sw. water.
Liriodendron tulipifera, L.	16	16	nil	Sw. water.
Lychnis coronaria, Desr.	43	100	nil	Sw. water.
Lychnis Githago, Scop.	43	100	nil	Sw. water.

* All from the same sample.

	Years old.	No. of Seeds.	Per cent. Germ.	
Marsdenia Leichardtiana, F.v.M.	13	50	nil	Sw. water.
Melia Azedarach, L.	20	100	nil	Sw. water.
Mucuna nivea, D. C.	15	6	nil	Sw. water.
Nelumbium speciosum, Willd.	13	2	nil	Sw. water.
Nicotiana glauca, R. Grah.	16	100	nil	Sw. water.
Panax elegans, C. Moore and F.v.M.	16	100	nil	Sw. water.
Panicum miliaceum, L.	16	100	nil	Sw. water.
ditto	16	100	nil	Sw. water.
Pastinaca sativa, L.	5	100	nil	Sw. water.
Phalaris canariensis, L.	15	100	nil	Sw. water.
Phaseolus Mungo, L.	14	100	17	Sw. water.
ditto	14	100	48	Sw. water.
Ranunculus bulbosus, L.	18	50	nil	Sw. water.
Raphanus sativus, L.	15	100	nil	Sw. water.
Ricinus communis, L.	3	19	47	Sw. water.
Sapindus (emarginatus), trifoliatus, L.	20	25	nil	Sw. water.
Saponaria Vaccaria, L.	20	25	nil	Sw. water.
Sequoia sempervirens, Endl.	16	100	nil	Sw. water.
Sequoia gigantea, Lindl. and Gord.	16	100	nil	Sw. water.
Sorghum sp. ?	16	100	5	Sw. water.
Sorghum sp. ?	16	100	nil	Sw. water.
Sorghum sp. (?)	16	100	15	Sw. water.
Spergula arvensis, L.	31	12	nil	Sw. water.
ditto	14	100	3	Sw. water.
Spergularia rubra, J. and C. Presl.	14	25	52	Sw. water.

	Years old.	No. of Seeds.	Per cent Germ.	
Sterculia, sp. ?	16	100	nil	Sw. water.
*Swainsona monticola, Benth.	18	61	nil	Sw. water.
ditto	18	39	59	Filed to produce swelling.
Syncarpia laurifolia, Tenore	16	60	70	Sw. water (used selected sample of well developed seeds)
Tristania conferta, R. Br.	16	100	nil	Sw. water
Triticum vulgare, Vill.	13	100	nil	Sw. water
ditto	6	100	nil	Sw. water
ditto	9	100	nil	Sw. water
ditto	10	100	nil	Sw. water
ditto	8	100	5	Sw. water.
ditto	7	100	nil	Sw. water.
ditto	2	100	nil	Sw. water, withered grains
ditto	9	100	3	Sw. water.
ditto	5	100	19	Sw. water
ditto	4	100	72	Sw. water.
ditto	3	100	nil	Sw. water.
ditto	5	100	52	Sw. water.
ditto	12	100	nil	Sw. water.
ditto	14	100	nil	Sw. water.
ditto	15	100	nil	Sw. water.
ditto	9	100	5	Sw. water.
ditto	6	100	92	Sw. water.
ditto	9	100	nil	Sw. water.
ditto	11	100	nil	Sw. water.
Tunica prolifera, Scop.	8	25	4	Sw. water.

* All from the same sample.

Structure of Seed Coat and Resistance to Water.

As has been already mentioned in the introduction to this paper, Dr. White examined more than 60 kinds of hard seeds, and found that in every case the seed was covered by a cuticle which rendered it impermeable to water. This cuticle stained brown with chlor-zinc-iodine.

In this work I have examined an additional number of seeds, and have selected six kinds, the seed coats of which differ somewhat in structure. These have been used throughout as types and subjected to various treatments, in order to determine the detailed microscopical structure of the impermeable coat or coats, the nature and distribution of the impregnating substances, and the character of the material forming the basis of the cuticularised walls. In each case a large number of seeds were soaked in water for forty-eight hours, and all those which remained unswollen after that time were picked out and set aside for further treatment. In the first place hand sections of the seed coats were cut and stained on the slide with chor-zinc-iodine, and in this way all the cuticularised portions were clearly differentiated. The thickness of the cuticle was also measured. The seeds were then treated with strong sulphuric acid for short periods of time, well washed and soaked in water for twenty-four hours between each application. As soon as a seed was seen to swell hand sections were cut, stained and examined, and compared with those of the untreated seeds. In this way it was possible to determine the smallest amount that must be removed from the coat to enable water to enter the seed, and hence to determine the internal boundary of the impermeable covering.

In many cases the water appeared to enter at one point, and gradually pass to the remaining portion of the seed, causing it to swell also. This was, of course, most noticeable in the larger seeds, and in such cases the sections were cut from the part that swelled first.

The seeds selected for this special treatment were *Indigofera arrecta*, *Cytisus albus*, *Acacia melanoxylon*, *Melilotus alba*, *Albizzia lophantha* and *Canna indica*.

Indigofera arrecta.—The minute structure of the coat of this seed has already been described by Bergtheil and Day, who found a membrane to be present which stained with phosphoric acid and iodine, but not with chlor-zinc-iodine, and thence concluded that the membrane was not the same as ordinary cuticle. With carefully prepared chlor-zinc-iodine, however, I was able to obtain the ordinary staining results for cuticle in this case, thus confirming Dr. White's results.[2]

The average thickness of the cuticle was 0.008 mm. The walls of the palisade cells below the cuticle stained the purplish colour of hemicellulose rather than the blue characteristic of cellulose. In treating with sulphuric acid the periods were short ones of five, ten, fifteen and twenty minutes. There is a considerable variation in the resistance of the seeds to the action of the acid. Some swelled after fifteen minutes, while others required thirty minutes or longer to make them permeable. Sections of the swollen seeds showed that the cuticle had been removed by the action of the acid, and that the ends of the palisade cells were exposed on the surface. (Fig. 79 [3].) Sections for comparison were also made from a more resistant seed, which remained unswollen in water after previous treatment with the sulphuric acid for a corresponding period. In this the cuticle was still visible as a continuous layer, but was reduced to about one-third of its original thickness. (Fig. 79 [2].) From this it is evident that the resistant powers of this seed are due to the cuticle only, and that the inner layer is as impermeable as the outer, and that the whole layer must be removed in order to allow water to pass readily through the palisade cells and to enter the seed.

Cytisus albus.—The structure of the coat (Fig. 79 [4]) is practically the same as that of *Indigofera arrecta* except that the cuticle is only half the thickness of that of the latter seed, being 0.004 mm. in thickness. The seeds were treated with sulphuric acid for five, ten, fifteen and twenty minutes. There was some variation in the length of treatment required, but the average time was fifteen minutes, which was shorter than the average for seeds of *Indigofera arrecta.* This was only to be

1 Ann. Bot., vol. xxi., Jan., 1907.
2 Proc. Roy. Soc. Victoria, vol. xxi., pt. i.

expected, as the seed coats were so much alike in structure, and those with a thicker cuticle naturally required longer treatment. Sections of the swollen seeds showed that the cuticle alone had been removed by the acid, and that the palisade cells were intact, so that in this type also it is the cuticle alone which confers impermeability on the seed.

Acacia melanoxylon.—These seeds are also covered by a layer of cuticle which is much thicker than in either of the preceding types, being 0.013 mm. (Fig. 79 [5].) The walls of the palisade cells are of cellulose, and not hemicellulose, as was the case with the majority of seeds that I examined. As this cuticle was thicker than the others the periods of treatment were corresponding longer, and it was found, instead of swelling in water after about fifty minutes, as might have been expected, it was only after one hour and twenty minutes that they became permeable. Microscopic examination showed, as in the previous cases, that the cuticle only was gone, and that the palisade cells were unchanged, so it seems safe to conclude that the cuticle is of a more resistant nature than that of Cytisus albus or of Indigofera arrecta.

Melilotus alba.—The seed coat in this case is of an entirely different type. The outer layer consists, like the others, of palisade cells covered externally by a structureless membrane which, however, did not appear to be cuticle but hemicellulose. It stained majenta with chlor-zinc-iodine. To confirm this result I tested similar sections with phosphoric acid and iodine, and also with chlorophyll, but in no case did the outer membrane give the cuticle reaction.

With regard to the palisade cells themselves the greater part of the wall appeared to be composed of hemicellulose, and the outer ends only were cuticularised and microscopic examination showed the outer cuticularised ends projecting, as it were, into the external hemicellulose membrane. (See Fig. 1 [6].)

In seeds which had been soaked in sulphuric acid for twenty minutes the outer membrane, and, in addition, the cuticularised ends of the cells were dissolved away. Such seeds swelled readily in water. In order to find whether the outer membrane was in itself impermeable to water, some more seeds were treated for shorter periods in order to dissolve off the outside

covering without directly affecting the palisade cells. Such seeds swelled in water, and microscopic examination showed that the ends of the palisade cells were quite intact, but had separated from each other, as shown in Fig. 79 [7]. From this it would appear that the outer membrane is instrumental in conferring impermeability on the seed, though not directly responsible for it, as is the case with a true cuticle. It seems probable that it serves rather as a cement substance, by means of which the cuticularised ends of the cells are held closely together, thus forming a barrier through which the water cannot penetrate, and as soon as the membrane is removed, the ends separate, and the water passes in between them.

In all the foregoing cases the treatment also took place at average room temperature—i.e., 12 to 15 deg. C.

Albizzia (Acacia) lophantha.—The seeds used for this test were not fresh material as was the case with the other five, but old seeds which had remained buried in the soil for at least twenty-three years. They proved to be remarkably resistant to the action of sulphuric acid, and to have retained their full power of germination. I obtained also some fresh seed of the same kind, for purposes of comparison, and this was not only considerably less resistant, but had a much lower percentage of germination. This is explained by the fact that, during the time the seeds remained buried in the soil all the non-germinable and less resistant seeds had either decayed or germinated, and those that were left represented the naturally selected good seeds of many seasons. The degree of resistance varied to an astonishing degree; some of the seeds swelled after one to five hours in acid, about 38 per cent. required 40 hours' treatment to make them swell, while 6 per cent. only swelled after an application of over 80 hours. The average temperature during the treatment was 12 deg. C.[1] I made a second test, keeping the acid at 30 deg. C., and found that about 80 per cent. swelled after $7\frac{1}{2}$ hours in acid, and the remainder at the end of $10\frac{1}{2}$ hours. This was the only case in which I tried the effect of using sulphuric acid of different temperatures. Many seeds,

1 According to Hiltner (Arbeiten aus der Biolog. Abteil f. Land w. Forstwirtsch am Kaiserl. Gesundheitsamte Bd. III., p. 29, 1903. "Seeds of A. lophantha required 10-15 hours in H_2SO_4 to make them permeable—no temperature mentioned."

even after prolonged treatment with the acid, remained unswollen in spite of the fact that a considerable amount of the outer coat had obviously been removed. The reason for this can be seen by reference to the figures of *A. lophantha*. Fig. 80 a shows the structure of the untreated seed coat. It was covered externally by a distinct cuticle 0.015 mm. in thickness; there were two layers of palisade cells instead of one, as is more usual. The cells were of varying lengths, those of the deeper row formed an undulating surface, over which those of the outer were moulded in such a way as to form a level surface on the exterior of the seed. The walls of the outer palisade cells were entirely cuticularised, and those of the inner also for some distance from the outer end, the remainder of the wall was of ordinary cellulose, and the cell contents were protoplasmic. The cuticularisation ended abruptly, and in the stained sections its limit was marked by a sharp line running across the cells. The lumina of the cuticularised portions of the cells are coloured black in the figure as they were otherwise difficult to define, but as far as could be seen they were quite empty. Fig. 80 b is a section of a seed which had been treated with acid for several hours, and which remained unswollen in water, although a good portion of the testa had evidently been removed. It can be seen that the outer palisade cells were almost corroded away, but as the inner cells were still intact, it was impossible for water to enter the seed. Fig 80 c is a section of a seed which had swollen in water, and shows that as soon as the walls of inner cells were corroded away as far as the openings of the lumina, water could then run into the cell cavity, pass through the inner cellulose wall and so enter the seed.

Canna indica.—This was the only hard seed I examined that had not a definite cuticular membrane covering the surface. The coat consisted of palisade cells, the walls of which were cuticularised except at the inner ends. Running transversely near the middle of the cells was a definite line which did not appear to have any morphological existence, or even to mark the boundary between layers of varying cuticularisation, but which was apparently the result of an optical effect. The lumina were narrow, with two dilatations, a slight one at the outer end, and

a large one at the inner end; the latter had protoplasmic contents.

The shape of the lumina gives the cells a very characteristic appearance. At intervals across the lumina were delicate oblique partitions of cuticularised substance. The seeds swelled after about two hours in sulphuric acid at 22 deg. C. The coats of the swollen seeds were very soft, and it was difficult to cut satisfactory sections of them. So I embedded them in paraffin without dehydrating, and cut sections with a horizontal microtome. The sections showed that the palisade cells were corroded away somewhat irregularly, so that it was difficult to judge to what extent the corrosion was necessary in order to permit water to enter, but it seems probable that it must at least proceed just beyond the last cross partition in any one cell. The entry of water into the cell would cause such a distortion of the micellae of cellulose as to give rise internal stresses and strains would disturb the micellae in other parts, so that they would become forcibly separated, and this would enable the molecules of water to push their way in. The results of the above experiments are summarised in the following table:—

Seed.	Thickness of Cuticle.	Average time in sulphuric acid at 12-15° C. required to produce sw.	Impermeable portion of Seed.
Indigofera arrecta -	0.008 mm.	15–30 min. -	Cuticle
Cytisus albus - -	0.004 mm.	15 min. - -	Cuticle
Acacia melanoxylon	0.013 mm.	1 hr. 20 min. -	Cuticle
Melilotus alba -	No true cuticle	10 min. - -	Cuticularised ends of palisade cells
Albizzia lophantha -	0.015 mm.	40 hrs. at 12° C. 7 hrs. at 30° C.	Cuticle and palisade cells
Canna indica - -	No cuticle	2hrs. - -	Palisade cells only

Nature of Cuticle and Methods of Softening Hard Seeds.

In the formation of cuticle the cell walls have become impregnated with cutin. Whether these materials penetrate the cell wall as a waxy substance, or whether they are deposited as the result of subsequent chemical change, is unknown. Van Wisselingh,[1] contrary to the opinions of Von Hohnel and Zim-

1 Archives Neerlandaises des Sciences Exactes et Naturelles, tome xxviii., 1894.

merman, concluded that cutin must always pass through cellulose to reach cuticle. In this it differs from cork in which the suberin is formed in direct contact with the protoplasm. The most important impregnating substances are compounds of glycerine with stearic, palmitic and suberic acids, and these, being of a fatty nature, should be soluble in ordinary fat solvents, saponified by potash, and should have a definite melting point. Associated with these fatty substances are other non-melting materials, and so intimate is the connection between the two that it is often difficult for the various reagents to act on one of the two impregnating substances, as its action may be hindered by the presence of the other. For example, a temperature of over 200 deg. C. may be required to cause physical decomposition of cutin, but once the materials are separated they will remain liquid at a much lower temperature. Possibly the cutin substances exist in the intact cuticle in a kind of loose chemical combination.

The work hitherto has been done with leaves of Oleander, Eucalyptus, Holly, etc., in which the cuticle, though well developed, cannot compare for thickness with that present on the outside of many hard seeds. Van Wisselingh in his work distinguishes between cuticle and thickened cuticularised cell walls. In the former he found only a trace of cellulose or none at all, while the cuticularised walls had a definite framework of cellulose. The cuticle in these leaves would seem therefore to be an exudation from the cells of the epidermis or a deposition of cuticular substances on the outside of the cell walls of such cells. It seems, however, highly improbable that a cuticle of the thickness found covering hard seeds should exist without a framework of some kind, but much more likely that it should be formed by the deposition of cutin within the substance of the original cell wall (or some modification of it), which, in consequence, would increase greatly in thickness. The particles of cutin would be evenly distributed among the micellae of the cellulose forming the framework.

If such seeds were treated with fat solvents the waxy materials should be dissolved out, and the insoluble basis be left, and it would be possible to detect the presence and nature of the latter by using suitable stains. If an outer membrane can be

shown still covering the seed, and the thickness of it be the same as that of the original cuticle, it would be safe to conclude that in the coverings of hard seeds at least an insoluble basis is present throughout.

The method used was as follows—the seeds were treated for varying lengths of time with chloroform, warm alcohol, turpentine, and strong caustic potash, sections were made of the seeds which swelled in water after the treatment, these were stained as before with chor-zinc-iodine and compared with similar preparations of untreated seeds. The seeds used were the same as those selected for the examination of the structure of the seed coat. Of the reagents used chloroform and caustic potash gave most satisfactory results, and turpentine had no effect whatever. Boiling absolute alcohol produced swelling in all cases, but as soaking in water or 70 per cent. methylated spirit at a corresponding temperature gave similar results it seems probable that the action is mainly a physical one, due to the melting of the fatty substances by the action of heat.

Indigofera arrecta and *Cytisus albus* gave similar results in all cases. Boiling in absolute alcohol for two hours produced swelling, and sections of the swollen seeds showed that the cuticle had entirely disappeared. One hour in boiling alcohol caused a few seeds to swell, and in these also the cuticle was gone, but there was a distinct rather ragged bluish line along the outer margins of the cells. As the sections dried this line seemed to shrink somewhat, although, as it was not very thick in the first place, the change was not remarkable. Maceration in chloroform at 30 deg. C. for eighteen weeks was also instrumental in producing swelling, and the stained sections of these seeds showed a marked contrast with those of untreated seeds. The outer membrane no longer appeared dark brown, but was tinged with the violet colour of hemicellulose. (See Plate 81, a, b.) A similar result was obtained by soaking the seeds in a saturated solution of caustic potash at 30 deg. C. for four weeks, or by boiling in the same for 5 min. The sections after this last method were unsatisfactory on account of the excessive softening of all the parts by the action of the potash, whereas prolonged soaking in chloroform appears to remove the cutin without affecting in the least the structure of the carbohydrate basis in which it is embedded.

Seeds of Acacia melanoxylon gave similar results as far as swelling was concerned. Three hours' treatment in boiling alcohol and in water at the same temperature caused a number of the seeds to swell on subsequently soaking in water, and the power of swelling was also restored by maceration for 18 weeks in chloroform at 30 deg. C. When, however, a comparison was made between stained sections of treated and untreated seeds, no difference could be detected between them, and both appeared as shown in Plate 81, c. The most probable explanations seemed that either there was some other material present in the outer wall which prevented the cellulose reaction or that the supporting tissue was some substance other than cellulose. If the former were the case the most likely material to mask the cellulose colour would be tannin. The seeds were therefore tested for this substance, with potassium cyanide, ammoniacal solution of ammonium picrate, lead acetate and caustic potash followed by sulphurous acid, and with ferric chloride,[1] and in each case the result was negative. The material which would be most likely to occur in the place of cellulose is pectose. According to Van Wisselingh pectose is somewhat like cellulose, but more gelatinous in character. In case the framework should be a mixture of pectose and cellulose, the sections were macerated in cuprammonia until the walls of the palisade cells, which were pure cellulose, were dissolved away; they were then stained with iodine and phosphoric acid, which stains pectose pale yellow, but as this colour did not differ much from that shown by the untreated seeds a further test was necessary. Following the directions of Van Wisselingh[2] for the removal of pectose the seeds were boiled repeatedly in dilute acids and alkalis, and washed between each operation, but this treatment softened the seeds to such an extent that section cutting was impossible. The second method of heating in glycerine from 250-300 deg. C. proved more satisfactory, and after half-an-hour's treatment the outer membrane was quite dissolved away (Plate III. d), so it seemed that in this case the framework of the cuticle was pectose and not cellulose or hemicellulose. This variation in the nature of the supporting

1 Methods given in Czapek Biochemie.

2 Czapek Biochemie, i., p. 552.

tissue is of interest because correlated with it, as already shown, is a greater resistance to the action of sulphuric acid.

The results obtained with Melilotus alba did not correspond with any of the others on account of the different nature of its seed coat. Boiling in absolute alcohol for five minutes or for three-quarters of an hour in 70 per cent. methylated spirits at 73 deg. C. produced swelling, but there was no apparent change in the structure of the seed coat. The swelling must have been due to the disturbance of the micellae of hemicellulose by the action of heat causing the cuticularised cells to become separated, and not to any actual change in the cuticularised substance. Hemicellulose, being unaffected by chloroform, effectually prevented the latter from reaching and dissolving out the fats in the cuticularised walls, and maceration in chloroform at 30 deg. C. for eighteen weeks had no effect whatever on the seeds. Sections were then made and kept in chloroform at the same temperature for a period of four weeks, and at the end of that time were washed and stained with chor-zinc-iodine. The whole cell wall now showed the hemicellulose colour, which was darker at the tip, where it had been previously cuticularised. (Plate 81 c.) The action of caustic potash was to entirely remove the outer membrane and to take out, probably by saponification, the waxy substances from the cuticularised parts.

In the case of *Albizzia lophantha* some of the tests were made with both old and new seed, and although the final results were the same, the degree of resistance of the two samples differed widely. For example, two hours in boiling alcohol caused 69 per cent. of the fresh seeds to swell, whereas three hours were required to produce a similar result with the old seed. As in all other cases the alcohol had no apparent effect on the structure of the cuticularised parts. In using caustic potash, four weeks at 30 deg. C. or one hour on a boiling water bath were required to produce swelling. The seeds' coats were softened to such a degree that section cutting was out of the question, but parts mounted and stained with chlor-zinc-iodine gave a distinct hemicellulose reaction. Maceration in chloroform for 18 weeks at 30 deg. C. also produced swelling, and in prepared sections the palisade cells which had previously stained

brown showed the purplish colour of hemicellulose (Pl. 81 f, g).

Canna indica served to confirm the above results. After 9 weeks in chloroform one out of ten swelled, and the seed coat gave the cellulose reaction, and a section of such a coat stained with chlor-zinc-iodine is figured in Pl. 81 h.

Swelling was also produced by the action of caustic potash, but seeds required long maceration in this substance to produce satisfactory results—1 hour in test tube on boiling water bath, and over 3 months at 30 deg. C.

Summary.

1. The general result of the series of germination tests in the first part of this paper confirms Prof. Ewart's statement that the macrobiotic seeds belong for the most part to the order Leguminosae, that the highest percentage of germination occurs among cuticularised seeds, and that the more impermeable the cuticle the higher is the percentage of germination. This fact is demonstrated by the results obtained with *Acacia acinacea*, *A. decurrens* and *A. pycnantha*, from these samples those seeds which swelled in water showed a feeble power of germination, while hard seeds from the same sample, which required prolonged treatment with sulphuric acid to make them swell, showed a percentage of germination from fifty to one hundred. There were a few exceptions to the above, which are worthy of note. *Eucalyptus calophylla* and *E. diversicolor* both possess macrobiotic seeds although unprovided with any specially impermeable coverings; they are further remarkable, as longevity is not as a rule a characteristic of large seeds containing oil. Another interesting result was obtained in the case of *Sorghum*, in which one sample sixteen years old showed fifteen per cent. of germination. This places *Sorghum* above *Triticum*, which is one of the longest lived of the cereals.

2. The impermeability of hard seeds in all those examined is due to the presence of cutin, which may—(a) form a membrane on the outside of the seed as in *Cytisus albus*, *Indigofera arrecta*, and *Acacia melanoxylon*, (b) be laid down in the cell walls of the palisade cells as in *Melilotus alba* and *Canna indica*, or (c) be found both as an outer membrane and in the walls

of the palisade cells as well, as in *Albizzia lophantha*. The degree of impermeability does not depend only on the thickness of the cuticle, but probably on the proportion of waxy substance present in the membrane.

3. The cuticle found covering hard seeds differs somewhat from that existing on many leaves. In the former it is an exudation beyond the cell wall, whereas in the latter it consists of a definite basis throughout which the particles of cutin are deposited. In a thick cuticle like that of *Albizzia lophantha* the greater thickness is probably due, in part at least, to a greater proportion of cutin being present, so that the degree of separation of the micellar basis must be greater than in a thinner cuticle like that of *Cytisus albus* or *Indigofera arrecta*. The nature of the substance forming the basis may vary somewhat. Judging from the seeds examined it seems, in the majority of cases, to be hemicellulose; in *Canna indica* it seemed intermediate between cellulose and hemicellulose, and in one case (*Acacia melanoxylon*) it was made up of pectose, and in this last case the cuticle was, curiously enough, of a more resistant nature than that of those which had a basis of hemicellulose.

EXPLANATION OF PLATES. LXXIX.—LXXXI.

Plate LXXIX.

Fig. 1.—Seed coat of Indigofera arrecta.
Fig. 2.—Same after short treatment with sulphuric acid.
Fig. 3.—Same after longer treatment with sulphuric acid.
Fig. 4.—Seed coat of Cytisus albus.
Fig. 5.—Seed coat of Acacia melanoxylon.
Fig. 6.—Seed coat of Melilotus alba.
Fig. 7.—Same after treatment with sulphuric acid.

Plate LXXX.

Fig. a.—Seed coat of Albizzia lophantha.
Fig. b.—Same unswollen after treatment with sulphuric acid.
Fig. c.—Same swollen after prolonged treatment with H_2SO_4.
Fig. d.—Seed coat of Canna indica.

Plate LXXXI.

All sections stained with chlor-zinc-iodine.

Fig. a.—Seed coat of Indigofera arrecta after maceration in chloroform.

Fig. b.—Similar sections of Cytisus albus.

Fig. c.—Seed coat of Acacia melanoxylon apparently unchanged by maceration in chloroform.

Fig. d.—Same after treatment with glycerine at 200 deg.

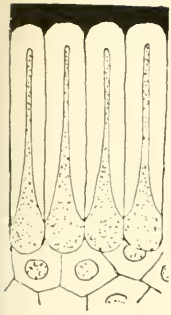
Fig. e.—Seed coat of Melilotus alba after section treated with chloroform.

Fig. f.—Seed coat of Albizzia lophantha (untreated).

Fig. g.—Same after maceration in chloroform.

Fig. h.—Seed coat of Canna indica after maceration in chloroform.

The coloured plate accompanying this paper was originally printed in the "Journal of Agriculture," and I have to thank the Hon. G. Graham, M.L.A., Minister of Agriculture, for the gift of copies of the plate for inclusion in the present volume.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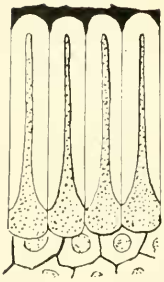
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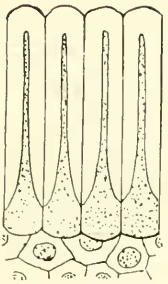
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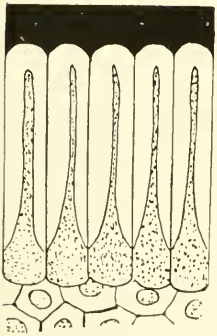
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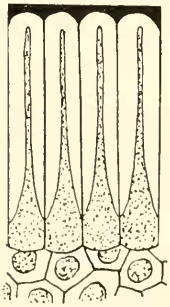
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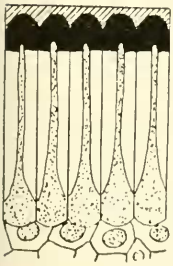
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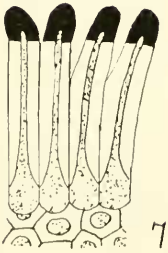
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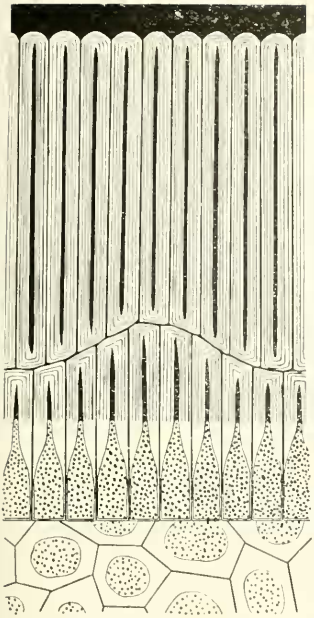
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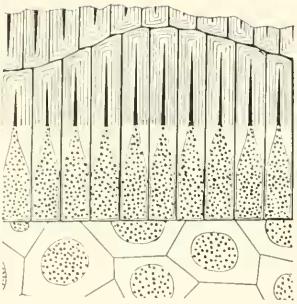
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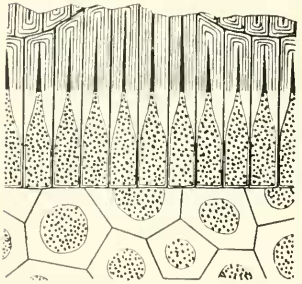
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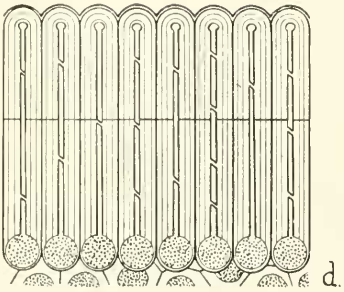
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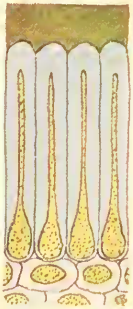
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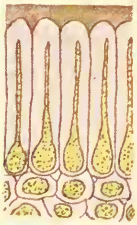
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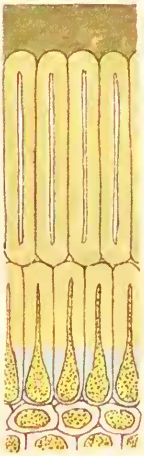
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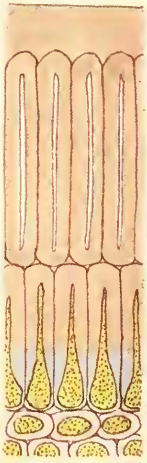
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h.

Bertha Rees, Del

SECTIONS OF SEED COATS OF HARD SEEDS.